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Insects for biodiesel production

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ABSTRACT

In this paper, the fat content of insects is studied for its utilization in the production of biodiesel. The study has shown the great fat potential of insects, highlighting a large number of species with an ether extract higher than 25%, including a large number in excess of 30% and some even reaching levels close to or above 77%. Moreover, a review of the main criteria to be considered for the selection of insect species for biodiesel production is carried out. It was observed that the fat content varies widely between orders, species, and their stages of development – larva, prepupa, pupa, nymph or adult – with the larval stage being that at which the most fat is accumulated. Furthermore, variations in the fat content were observed within the same species due to factors such as origin (wild or bred in captivity) or type of diet. This last factor is one of the most important to take into account for the selection of insect species with the objective of using their fat in the production of biodiesel. The principal conclusion of this study is that insects, through the development of their life cycle, can be fed with agricultural, industrial or urban by-products in order to accumulate a large amount of fat with potentially excellent quality (fatty acids C16–18), for conversion into energy through biodiesel production. Moreover, the resulting protein can also be used as a protein source in animal feed. Therefore, insects are a renewable source of protein and energy.

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Contents

1. Introduction

The world is confronted with the twin crises of fossil fuel depletion and environmental degradation [1]. Fuels are the world's main energy resource and are considered the centre of energy demands [2]. Most of the great powers have been convinced of the need to develop clean (zero-emissions) and renewable (infinite) energies in order to replace fossil fuels, and to help achieve a development

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that is harmonious, balanced, and respectful to the environment. The depleting reserves of fossil fuel, increasing demand for diesels and uncertainty in their availability are considered to be the important triggers for many initiatives to search for an alternative source of energy that can supplement or replace fossil fuels [3]. Renewable energy sources have several advantages, such as the reduction in dependence on fossil fuel resources and the reduction in carbon emissions to the atmosphere [4].

Biodiesel is defined as the mono-alkyl esters of long-chain fatty acids derived from renewable feedstock, such as vegetable oil or animal fats, for use in compression–ignition engines [5]. The advantages of biodiesel as diesel fuel are its portability, ready availability, renewability, higher combustion efficiency, lower sulphur and aromatic content, higher cetane number and higher biodegradability [6].

In general, biodiesel feedstock can be categorized into four groups: vegetable oils (edible or non-edible oils), animal fats, used cooking oil including triglycerides and recently oleaginous microorganisms such as microalgae, fungi, bacteria and yeast [7].

Ninety-five percent of global biodiesel production is made from edible vegetable oils [8], and the cost of these raw materials accounts for 60–75% of the total cost of biodiesel fuel [7,9]. Since the prices of edible vegetable oils are higher than that of diesel fuel, waste vegetable oils and non-edible crude vegetable oils are preferred as potential low-priced biodiesel sources [3]. However, should vegetable oil processing costs be reduced through increased plant capacities, it would become a more viable alternative to diesel fuel [10].

Animal fats contain higher levels of saturated fatty acids, and are therefore solid at room temperature [11]. Animal fats used to produce biodiesel include tallow [12], choice white grease or lard [11], chicken fat [12], yellow grease [13] and fish oil [14]. Compared to plant crops, these fats frequently offer an economic advantage because they are often priced favourably for conversion into biodiesel [3].

The utilization of used cooking oil can lower the cost of feedstock significantly. Its price is 2–3 times cheaper than virgin vegetable oils [15]. Therefore, studies are needed to find a cheaper way to utilize used cooking oils to make biodiesel fuel [5]. However, the quality of used cooking oils can be bad [16].

Oleaginous microorganisms are defined as microbes with microbial lipid content in excess of 20% [7]. Although there are all kinds of microorganisms that store oils, such as microalgae [17,18], bacillus, fungi and yeast, not all of them are available for biodiesel production [7].

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources. The remaining challenges are its cost and limited availability of fat and oil resources [5]. There are two aspects of the cost of biodiesel: raw material (fats and oils) and processing. At the industrial level, biodiesel is currently produced from plant and animal oils, and though its potential for feedstock has been studied, production from insects is not significant.

Apparently, there is a lack of scientific papers considering insects in the production of biodiesel. However, EcoSystem Corp. recently asked the U.S. Department of Energy for $1.75 million to finance the study of a new production scheme for biofuels: rearing fly larvae with food wastes, and transforming the fat they produce into fuel [19]. Currently, they produce an oil under the title of EcoSystem's MAGOIL™, which is sent to Biofuel Industries Group in Michigan [20].

On the other hand, aquaculture depends largely on fish meal as a protein source. This makes the breeding of fish in captivity depend on wild fish catch. To help solve this problem, the introduction of insect meal as an alternative source of protein is being successfully researched. But insects studied have more fat than necessary in fish diet, thus this fat surplus can be used for biodiesel production. Therefore, the use of insects could ensure a steady production of protein and fat, both in quantity, quality and price.

Currently it is difficult to determine the costs of intensive insect production, since, except for pollination and biological control; they have only been produced for experimental purposes. The lack of demand for this product has prevented its implementation on an industrial scale, thus it is not easy to establish real and competitive prices. As a guideline, in Asia, where the use of flour silkworm (*Bombyx mori*) is widespread, the price per ton ranges from $ 350 to 1000 USA.

Moreover, certain species of saprophagous insects naturally contribute to the recycling of organic matter, thus knowing their biological needs; they can be used to degrade organic waste and to obtain an economically viable biomass for animal feed. Insect breeding takes place in warehouses, so there is no need of large land areas such as in the case of energy crops, or water areas, as in the case of microalgae, especially when compared to crops such as soybeans. These two aspects make highly populated countries such as China unable to direct their acreage to energy crops, unlike USA or Brazil, since they must devote their acreage to food crops. All these reasons make the mass rearing of insects have a viable future, and it arises, a priori, as an environmentally sustainable technology.

2. A brief overview of insect utilization

Insects belong to the phylum Arthropoda. At present, there are around one million known species in this group of animals, although it has been estimated that their global diversity is as high as 80 million [21]. Grimaldi and Engel [22] suggest that only about 20% of insects have been named and described. More than 58% of the known global biodiversity are insects [23]. This fact greatly contrasts with the vertebrates, which only constitute 3% of the total diversity. The greatest concentration of insect species is found in tropical areas. However, they are present around the world, with the exception of marine habitats which contain few insect representatives but include a high biodiversity of other arthropod groups such as Crustacea.

Insects have played an important role in the history of human nutrition in Africa, Asia and South America [24]. Today, there are over 1400 insect species consumed by humans on the planet [25]. Some of the most important groups are grasshoppers (Orthoptera), beetle larvae (Coleoptera), termites (Isoptera), different types of Hymenoptera (bees, wasps, ants), as well as cicadas and other aquatic hemiptera. With the consumption of insects, human beings mainly seek to cover the protein deficiency of their diet. Insects have protein content similar to flesh from fish, meat from mammals, birds and other vertebrates. Therefore, they are not only used as food in times of scarcity but are included in the diet, in a planned manner, throughout the year or in the seasons during which they are available [26]. So, insects offer great potential for application at both a nutritional and a commercial level.

3. Mass-rearing of insects for biodiesel production

Traditionally, the mass-rearing of insects has been focused on the production of the so-called “beneficial insects”, mainly in the agricultural field. In particular, the vast majority of the currently developed biofactories are focused on the production of natural enemies of agricultural pests, specifically, predators and parasitoids. Mass-rearing has also focused on control of species with medical-veterinary importance, specifically developing the sterile-male control technique (Sterile Insect Technology), which is currently also applicable to other types of pests. The basic principle of this technique is the large-scale mass production of sterile

males of a specific pest, which are subsequently released in the wild. The purpose is to have a much higher number of sterile males than wild males, so that the development of the pest is reduced to a manageable level. At a smaller scale, protocols for mass production of insects are being developed with other purposes such as pollination, use in pet food or even as fishing baits.

An appropriate selection of species could be used as a source for biodiesel production, applying part of the current extensive knowledge concerning the artificial mass-rearing of insects. The main points to take into consideration regarding this type of rearing are as follows:

- Selection of strains or specific varieties adapted to artificial mass-rearing.
- Independent maintenance of egg-producing colonies assigned to the mass-rearing of larvae.
- Knowledge of the biotic and abiotic factors necessary to allow the controlled mass larval development.
- Development of quality control mechanisms for the process (both larval development and selection of egg-producing colonies).
- Optimization of the larval development medium (homogenization, characterization).
- Development of machinery and devices related to larvae/pupae harvesting.
- Development of devices for harvesting eggs.
- Larval development medium with traceability and certain physicochemical characteristics.
- Proper cleaning systems and disease prevention.
- Control systems for temperature and environmental humidity, ambient light quality and photoperiod.

4. Fat potential of insects

Insects often establish metabolic reserves [27,28], especially during the immature stages (larva, pupa, nymph) as they are necessary during certain non-feeding periods of their life cycle (e.g. diapause, metamorphosis, etc.). In the spaces between organs, insects possess a structure called "fat body" – a nutrient storage system used in the molting process (or metamorphosis) [29]. The fat body plays a major role in intermediary metabolism and it is the central storage depot of nutrients and energy reserves [30]. Whereas many insect tissues have vertebrate analogs, the fat body is an organ unique to insects [31]. The fat body is a relatively large organ distributed throughout the insect body, preferentially underneath the integument and surrounding the gut and reproductive organ [32]. It is the main organ involved in the metabolism of energy [33], and is involved in multiple other metabolic functions as well. Although lipids are one of the main metabolic reserves for the development from larva to adult, glycogen and proteins associated with body fat or proteins in the hemolymph are also stored [34].

The change in body fat during insect metamorphosis is called remodeling. Liu et al. [35] have found that fat cells dissociate slightly in each larval molt and re-associate soon after molting. However, this process of dissociation of body fat cells is much more dramatic during the larval-pupal metamorphosis.

Accumulation of lipids by diapausing insects is well documented [36]. In the order Diptera, diapause in adults is often associated with large body fat stores [37]. As an example, Adedokun and Denlinger [38] found that diapause larvae of *Sarcophaga crassipalis* contained almost twice the amount of extractable lipids and proteins in the hemolymph as non-diapause larvae. However, Saunders [39] observed in *Calliphora vicina*, that the proportion (in percentage of dry weight) of fat was similar between diapause and non-diapause larvae, though the fat content of this species was reduced by 30–40% during diapause [40]. These studies also suggest that an increased accumulation of fat is not part of the diapause syndrome in *C. vicina*, even though lipids must constitute a major metabolic store for the development from larva to adult, regardless of whether or not this development includes a diapausing phase.

According to the FAO [41], each 100-g measure of dried caterpillars contains about 53 g of proteins, 15% fats and about 17% carbohydrates. Their energy value is around 430 kcal per 100 g. However, as it is shown in Tables 1–6, the crude fat content varies widely between different insect orders and species. Moreover, once the insect oil is extracted, the resulting paste would be rich in animal protein that could easily be used in human or animal food (aquaculture, poultry or pigs).

Fat contents of insects studied so far are summarized in Tables 1–6, grouped by the order to which they belong. Tables also show the provenance of the insect – wild or bred in captivity – and the insect development phase – larva, prepupa, pupa, nymph and adult. Firstly, insects are sorted by the order to which they belong; secondly, alphabetically by name and, thirdly, by the fat content in % of dry matter. When there are different data for a species from various authors, these species have been ordered from lowest to highest fat content, regardless of their development phase.

As it can be seen, the data are quite varied: There are species with a fat content of 1.5% (*Oryctes boas*, order Coleoptera) or 2% (*Brachytrypes spp*, order Orthoptera), a large number of others with a larval-stage ether extract higher than 25%, another large number above 30%, others with 60% (*Galleria mellonella*, order Lepidoptera) and others with up to 77% (*Phasus triangularis*, order Lepidoptera). Fat not only varies between orders or species, but even within the same species; for example, see *Musca domestica*, in the order Diptera, which varies from 8.1 to 32.6%. But, it seems clear that adults have a lower proportion of body fat than larvae. It is known that the type of food ingested by insects is one of the most important factors affecting body composition; for example, the *G. mellonella* larvae (60% fat) basically feed on beeswax. Newton et al. [51] have found that the *Hermetia illucens* larvae that were fed with poultry manure contained 34.8% fat, but those that were fed with pig manure only contained 28.0%.

The reasons for this heterogeneity, apart from the aforementioned food consumption, are very diverse. It should be noted that insects are an animal group with a very long evolutionary history – much longer than mammals, for example – and furthermore that their adaptive potential allowed them to exploit a huge number of different ecological niches, resulting in specific, and often extreme, physiological adaptations. Furthermore, the postembryonic development of insects is also quite varied, including groups with an almost direct development (Ametabolism) from egg to adult, and others with varied metamorphic processes (Holometabolism vs. Heterometabolism). In particular, in animals with holometabolous development, the transition from larva to pupa occurs through a complete transformation at physiological and histological levels. In fact, larvae and adults often have completely different lifestyles, with the larval stages being responsible for the processing of food sources into biomass and achieving the transformation into adulthood (mainly related to reproductive tasks). As a result, in most cases, larvae are the ones that usually accumulate a higher lipid percentage, though the content in adults of some species can also be important.

Moreover, insects present the whole spectrum of possible feeding behaviours, including varieties such as predators, filter feeders, suspension feeders, and necrophagous, coprophagous, xylophagous, phytophagous, fungivorous, and saprophagous species; there are even many omnivorous species and several with different diets in their larval or adult stages. The characteristics of their life cycle are also very important since many species can remain in states of inactivity, during which they do not feed (e.g.

Table 1
Lipid content of different insect species of order Coleoptera.

Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Analeptes trifasciata	Larva	W	18.4	[26]
Aplagiognathus sp.	Larva	W	36.9	[42]
Aplagiognathus spinosus		W	36.0	[43]
Aplagiognathus spinosus	Larva	W	37.1	[42]
Apomecyna flavovittala	Larva	C	35.2	[44]
Apriona germari	Larva	C	41.5	[44]
Aromia bungii	Larva	C	35.9	[44]
Arophalus rusticus	Larva	W	56.1	[42]
Arophalus sp.	Larva	W	56.8	[42]
Callipogon barbatum	Larva	W	34.0	[42]
Callipogon barbatus		W	34.0	[43]
Chalcophora sp.	Larva	W	53.7	[42]
Holotrichia oblita	Larva	C	29.8	[44]
Homolepta sp.		W	18.0	[43]
Homolepta sp.	Larva	W	18.4	[42]
Macrodactylus lileaticollis	Larva	W	11.7	[42]
Melolontha sp.	Larva	W	18.8	[42]
Metamasius spinolae	Larva	W	25.5	[42]
Oileus rimator	Larva	W	47.0	[43]
Oryctes boas	Larva	W	1.5	[26]
Pachymerus nucleorum	Larva	W	49.3	[42]
Passalus af. punctiger		W	44.0	[43]
Passalus punctiger.	Larva	W	44.0	[42]
Paxillus leachei	Larva	W	47.2	[42]
Phyllognathus excavatus	Adult	W	15.9	Own data
Phyllophaga sp.	Larva	W	24.0	[42]
Rhantus sp.	Larva	W	6.3	[42]
Rhynchophorus ferrugineus	Larva	C	13.8	Own data
Rhynchophorus palmarum	Larva	W	38.5	[45]
Rhynchophorus phoenicis	Larva	W	31.4	[26]
Rhynchophorus phoenicis	Larva	W	47.1	[46]
Scyphophorus acupunctatus	Larva	W	50.9	[42]
Scyphophorus acupunctatus		W	52.0	[43]
Strataegus aloeus	Larva	W	17.0	[42]
Tenebrio molitor	Adult	C	14.9	[47]
Tenebrio molitor	Adult	C	18.4	[48]
Tenebrio molitor	Pupa	W	36.6	[42]
Tenebrio molitor	Pupa	C	30.8	[48]
Tenebrio molitor	Pupa	C	41.5	[44]
Tenebrio molitor	Larva	C	30.1	Own data
Tenebrio molitor	Larva	C	32.8	[48]
Tenebrio molitor	Larva	C	35.2	[47]
Tenebrio molitor	Larva	W	38.2	[42]
Tenebrio sp.	Larva	C	55.1	[48]
Trichoderes pini	Larva	W	36.3	[42]
Zophoba morio	Larva	C	38.0	Own data
Zophoba morio	Larva	C	42.0	[47]

W, wild; C, bred in captivity.

Table 2
Lipid content of different insect species of order Hymenoptera.

Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Apis mellifera	Larva/adult	W	12.3	[26]
Apis mellifera	Larva	W	19.0	[43]
Apis mellifera	Pupa	W	20.0	[43]
Atta cephalotes	Reproductive	W	31.0	[43]
Atta mexicana		W	39.0	[43]
Brachygastra azteca		W	22.0	[43]
Brachygastra mellifica		W	30.0	[43]
Componotus sp		W	15.9	[49]
Melipona beeckeii		W	41.0	[43]
Myrmecosistus melliger	Reproductive	W	6.0	[43]
Oecophylla longinoda		W	41.3	[49]
Polistes instabilis		W	62.0	[43]
Polybia occidentalis bohemani		W	19.0	[43]
Polybia occidentalis nigratella		W	28.0	[43]
Polybia parvulina		W	21.0	[43]
Polybia sp.	Adult	W	13.0	[43]
Trigona sp.	Reproductive	W	41.0	[43]
Vespula squamosa		W	22.0	[43]

W, wild; C, bred in captivity.

Table 3
Lipid content of different insect species of order Orthoptera.

Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Acheta domestica	Adult	C	13.8	[48]
Acheta domestica	Nymph	C	14.4	[47]
Acheta domestica	Nymph	C	15.8	Own data
Acheta domestica	Adult	C	22.1	[47]
Brachytrypes spp.	Adult	W	2.3	[26]
Cytacanthacris naeruginosus	Adult	W	3.5	[26]
Heteracris littoralis	Adult	W	8.8	Own data
Locusta migratoria	Nymph	C	28.5	Own data
Melonoplus mexicanus	Adult/nymph	W	7.0	[43]
Plectotettia nobilis	Adult/nymph	W	7.0	[43]
Schistocera sp	Adult/nymph	W	17.0	[43]
Sphenarium histrio	Adult/nymph	W	4.0	[43]
Sphenarium purpurascens	Adult/nymph	W	11.0	[43]
Sphenarium sp	Adult/nymph	W	4.0	[43]
Sphenarium spp	Adult/nymph	W	12.0	[43]
Zonocerus variegatus	Adult	W	3.8	[26]
Zonocerus variegatus	Adult (winged)	W	28.0	[49]
Zonocerus variegatus	Nymph	W	31.0	[49]

W: wild. C: bred in captivity.

diapause phase, pupal phase, prolonged fasting, etc.), and adults of many species do not even need to feed at all during their lifetime. All these factors influence, obviously, the interpretation of the results of the previous table; the same species in different stages of development (larva, pupa, adult) or with different types of diet (phytophagy, saprophagy, omnivory) can present different values of fat content.

5. Main criteria for selecting insects for biodiesel production

Having seen the great potential for fat content in certain insect species, and the possibility of feeding them in many different ways, we are going to mention some criteria for selecting some species over others:

- Fat content (of larvae). As previously mentioned, fat content can be very variable throughout the life cycle of an insect. Particularly in holometabolous insects, large variations can exist not only between different immature stages (larvae vs. pupae) but also throughout the course of the larval development itself. For example, many dipterans are characterized by the existence of a mobile non-feeding prepupa stage. This type of larvae can have unique lipid concentrations. Moreover, metamorphic changes during pupal development usually involve a great biochemical diversity that can also affect fat content.
- Speed of completion of the life cycle. The life cycles of a large majority of insects are very fast, particularly for species that feed from decaying organic matter (saprophagous, necrophagous and coprophagous species). This is due to the ephemeral nature of this type of habitat. Even species with other ways of life (e.g. phytophagous species) have very fast growth rates compared, for example, to vertebrates. This is possible because of the combination of their small size and high metabolic rates. Many insects can develop their complete life cycle in just over a week, though at least 30 days are usually necessary to complete the cycle.
- Requirements of space and reproductive capacity. Space requirements of many insect species are also reduced in comparison to other animal groups. Because of their small size, a large number of specimens can be gathered in limited spaces for artificial rearing.

Table 4
Lipid content of different insect species of order Diptera.

Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Aedes sp.	Larva	C	16.1	[48]
Chironomus sp.	Adult	C	9.7	[48]
Copestylum anna	Larva	W	31.0	[43]
Copestylum haagii	Larva	W	31.0	[43]
Drosophila melanogaster	Pupa	C	10.5	[48]
Drosophila melanogaster	Adult	C	12.6	[48]
Drosophila melanogaster	Larva	C	29.4	[48]
Eristalis tenax	Larva	C	5.8	Own data
Hermetia illucens	Larva	C	28–34.8	[51]
Hermetia illucens	Prepupa	C	15.5	Own data
Hermetia illucens	Larva	C	18.0	Own data
Hermetia illucens	Larva	C	18.8	[50]
Lucilia sericata	Larva	C	16.5	Own data
Lucilia sericata	Pupa	C	25.7	Own data
Musca domestica	Larva	C	8.1–13.5	[52]
Musca domestica	Pupa	C	15.8	[48]
Musca domestica	Larva	C	20.0	[48]
Musca domestica	Larva	C	20.3	Own data
Musca domestica	Larva	C	20.7–25.3	[53]
Musca domestica	Larva	C	25.3	[53]
Musca domestica	Pupa	C	32.6	Own data
Protophormia terraenovae	Pupa	C	23.6	Own data
Protophormia terraenovae	Larva	C	28.3	Own data

W, wild; C, bred in captivity.

Table 5
Lipid content of different insect species of order Lepidoptera.

Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Aegiale hesperiaris		W	30.0	[43]
Anaphe infracta	Larva	W	15.2	[26]
Anaphe recticulata	Larva	W	10.2	[26]
Anaphe venata	Larva	W	23.1	[26]
Arsenura armida		W	8.0	[43]
Ascalapha odorata		W	15.0	[43]
Antheraea pernyi	Adult	C	34.5	[44]
Antheraea pernyi	Pupae	C	31.3	[44]
Bombyx mori		W	35.0	[43]
Bombyx mori	Larva	W	8.0	[47]
Catasticta teutila		W	19.0	[43]
Cirina forda	Larva	W	12.2	[54]
Cirina forda	Larva	W	14.2	[26]
Corcyra cephalonica	Larva	*C*	43.3	[44]
Eucheria socialis		W	16.0	[43]
Galleria mellonella	Larva	C	46.4	[48]
Galleria mellonella	Larva	W	60.0	[47]
Heliothis zea		W	29.0	[43]
Hylesia frigida		W	10.0	[43]
Latebraria amphipyrioides		W	7.0	[43]
Ostrinia nubilalis	Pupa	C	17.0	[48]
Ostrinia nubilalis	Larva	C	17.2	[48]
Ostrinia nubilalis	Larva	C	46.8	[44]
Pectinophora gossypiella	Larva	W	49.9	[44]
Phasus triangularis	Larva	W	77.0	[43]
Xyleutes redtembacheri		W	48.0	[43]

W, wild; C, bred in captivity.

If the biotic and abiotic requirements are maintained, their life cycles can last indefinitely over many generations, independently of external conditions.

- Less expensive feeding. Omnivorous decomposer species can be fed with a great variety of by-products and organic waste from sources such as food, animals and vegetation. Of particular interest is the possibility of using certain coprophagous species capable of feeding on dung and droppings of different types of livestock in intensive production. Necrophagous species could be fed with castoff material of slaughterhouses not intended for human consumption, and certain others could be fed with organic waste derived from restaurants/catering services, etc.

We can highlight, as an example of an insect species with great potential in biodiesel production, the larvae of *H. illucens* (Diptera: Stratiomyidae), also called Black Soldier Fly (in the Anglo-Saxon world). This insect currently presents a worldwide distribution from about 46°N latitude to 42°S latitude [56], including the regions of Australia, India, Africa and Europe [57]. This species is associated to saprophagous environments such as manure [50]; therefore, it is proposed to use manure as a larval substrate, and the insect biomass obtained to produce a fodder of potentially high quality [58–61]. Fig. 1 shows an example of these insects feeding pig manure. One advantage of this system is that, simultaneously with the degradation of organic waste, a large amount of animal biomass is obtained that can be used to create biodiesel or animal feed. Another advantage is that these larvae modify the manure microflora, reducing the presence of harmful bacteria [62].

The Black Soldier fly potentially presents a simple mass-production system. This is due to the migratory nature of their larvae, which pupate away from the source of development, allowing an effective separation of the decomposed matter. It is in this stage (prepupa) that they have their largest size, with a large store of fat. Migrant prepupae do not feed. Pupation lasts at least 10 days,

Table 6
Lipid content of different insect species of several orders.

Order	Species	Development phase	Provenance	Lipid content % (dry matter)	Reference
Blattodea	*Periploneta americana*	Adult	W	21.5	[49]
Blattodea	*Periploneta americana*	Adult	C	28.4	[48]
Hemiptera	*Acantocephala declivis*	Nymph	W	45.0	[43]
Hemiptera	*Corisella mercenaria*	Adult	W	9.0	[43]
Hemiptera	*Corisella spp.*	Egg	W	7.0	[43]
Hemiptera	*Edessa petersii*	Adult/nymph	W	42.0	[43]
Hemiptera	*Edessa sp.*	Adult/nymph	W	54.0	[43]
Hemiptera	*Euschistus egglestoni*	Adult/nymph	W	45.0	[43]
Hemiptera	*Pachilis gigas*	Adult	W	19.0	[43]
Hemiptera	*Pachilis gigas*	Nymph	W	26.0	[43]
Homoptera	*Hoplophorion monograma*	Adult	W	14.0	[43]
Homoptera	*Proarna sp.*	Adult	W	4.0	[43]
Homoptera	*Umbonia reclinata*	Adult	W	33.0	[43]
Isoptera	*Macrotermes bellicosus*	Adult (winged)	W	28.2	[26]
Isoptera	*Macrotermes bellicosus*	Adult (winged)	W	36.1	[55]
Isoptera	*Macrotermes nigeriensis*		W	28.3	[49]
Isoptera	*Macrotermes notalensis*	Adult (winged)	W	22.5	[26]
Isoptera	*Microtermes sp*		W	17.0	[49]
Megaloptera	*Chauliodes sp.*	Adult	C	19.5	[48]

W, wild; C, bred in captivity.

Fig. 1. Larvae of *Hermetia illucens* feeding pig manure.

and the larval feeding phase takes 3–4 weeks on average. Adults can carry out the laying of eggs without feeding, possibly thanks to the accumulation of fat stored during the larval stage.

6. Biodiesel production from insects

The biodiesel production from insects has been described by several authors [63–65]. In a 100 ml reactor equipped with a reflux condenser, a thermometer, a mechanical stirrer, and a sampling outlet. Biodiesel production was accomplished using a two step process: acid-catalyzed esterification of free fatty acids (FFA) (to decrease the acidity of the crude fat), and alkaline-catalyzed transesterification.

6.1. Acid-catalyzed esterification

The acid-catalyzed esterification step was a pretreatment used to convert free fatty acids in the crude fat into biodiesel, and to decrease the acidity of the crude fat. Specifically, 16 sets of 30 g of crude fat were pretreated to esterify the free fatty acid with methanol using 1% H_2SO_4 (w/w) as the catalyst at the following conditions: four sets were pretreated (methanol to fat ratio 8:1; time 1 h) at a temperature of 55 °C, 65 °C, 75 °C or 85 °C, respectively; four sets were pretreated (temperature 75 °C, time 1 h) with a methanol to fat ratio of 6:1, 8:1, 10:1, or 12:1, respectively; four sets were pretreated (methanol to fat ratio 8:1, temperature 75 °C) with a reaction time of 30 min, 60 min, 90 min, or 120 min, respectively. During esterification, 3 ml samples were withdrawn periodically to determine the free fatty acid conversion. After pretreatment, the reaction mixture was poured into a funnel, and was allowed to separate by gravity. The upper layer (crude fat and biodiesel) was then transferred to a reactor for alkaline-catalyzed transesterification.

6.2. Alkaline-catalyzed transesterification [66,67]

The upper layer (crude fat and biodiesel) obtained from the above acid-catalyzed esterification was mixed with methanol (methanol to fat ratio of 6:1) and the catalyst NaOH (0.8%, w/w). This mixture was placed in a 65 °C water bath for 30 min, with agitation by a magnetic stirrer. After the reaction, the mixture was separated by gravity. The upper biodiesel layer was then separated from the lower layer and purified by distilling at 80 °C to remove the residual methanol.

Table 7
Yields of biomass, crude fat and biodiesel for 1000 larvae of *Hermetia illucens* grown in 1000 g of manure, after 10 days at room temperature [63].

	Cattle manure	Pig manure	Chicken manure
Biomass (g)	127.6	207.4	327.6
Crude fat (g)	38.2	60.4	98.5
Fat yield (%)	29.9	29.1	30.1
Biodiesel (g)	35.6	57.8	91.4

7. Quality of insect fat for biodiesel production

Finally, this review study of insect fat and criteria for the selection and production of said insects would not be complete without taking into account the quality of the fat derived from them for biodiesel production.

Biodiesel is defined as a mixture of mono-acyl esters of animal and vegetable fatty acids [5]. The quality of the fuel depends on the amount and type of esters in the mixture, and the quality of each ester depends on the type of alcohol and fatty acid of which it is formed (sep biodiesel fatty acids).

Animal or vegetable fat is mostly made up of triglycerides. The use of triglycerides and their derivatives can be considered a viable alternative for biodiesel production [68]. Today, in fact, many vegetable sources such as the oil of sunflower, palm, soya or coconut are used as fuel. However, these sources have several drawbacks from the point of view of production and quality for use in biodiesel.

On the one hand, the production levels of these oils depend on bioclimatic conditions and require large areas of cultivation (20–40 GJ gross energy/ha/year and >0–10 GJ net energy/ha/year [69]) as well as a considerable amount of water.

Regarding quality, vegetable oils present important differences compared to conventional fuels. Their main disadvantages with respect to conventional diesel are (a) higher viscosity, (b) higher pour point, (c) higher flash point, (d) higher cloud point, (e) higher density and (f) the reactivity of unsaturated hydrocarbon chain [70].

Vegetable oils are generally subjected to several chemical processes for biodesel production. The most common of these are pyrolysis, microemulsification, dilution and transesterification [71,72]. Transesterification of natural oils and fats is the most commonly used method. Its aim is to lower the viscosity of the fuel. The transesterification reaction of triglycerides with alcohols (methanol, ethanol, propanol, butanol or amyl alcohol) can be catalyzed by alkalis, acids or enzymes [73,74]. The transesterification of triglycerides by supercritical methanol, ethanol, propanol or butanol has proved to be the most promising process [75,76].

The characteristics of animal fats differ significantly from those of vegetable oils, since they have more viscosity and higher pour and flash points [76]. Despite these negative properties, they are of strong interest because of their low price (they are generally waste from slaughterhouses or other industries). In fact, biodiesel has been obtained from salmon oil [77] and other animal fats [78,79]. One of the major problems associated with the use of biodiesel is poor low temperature flow properties, indicated by relatively high cloud points (CP) and pour points (PP) [80]. On the other hand, the utilization of inexpensive and easy to produce additives can eliminate these disadvantages [80]. Numerous, usually polymeric, additives were synthesized and reported mainly in the patent literature to have the effect of lowering PP or sometimes even CP. For the purpose of biodiesel improvement, organic based magnesium, molybdenum, manganese and nickel additives were synthesized, dosed into the tall oil methyl ester and performance and emission data were recorded with the additives [81,82]. Also, four metallic type additives have been studied in order to improve diesel fuel [83].

Table 8
Properties and qualities of biodiesel produced from different feedstocks in comparison with standard EN14214 and standard ASTM D975.

Fuel properties	Unit	ASTM D975	EN 14214	*Hermetia Illucens* (insect) [63]	Rapeseed [63]	Microalgal [99]	Soybean [99]
Density 15 °C	kg/m^3	880	860–900	885	880	–	913.8
Viscosity at 40 °C	mm^2/s	1.9–6.0	3.5–5.0	5.8	6.35	4.624	4.039
Sulphur content	wt.%	0.05	10[a]	–	<0.01	0.6[b]	0.8
Ester content	%	–	≥96.5	97.2	n/a	–	
Water content	mg/kg	<0.05	500[a]	0.03	0.03	<0.005	<0.005
Flash point (Fp)	°C	100–170	>120	123	n/a	>160	254
Cetane number		≥47	≥51	53	45	–	37.9
Acid value	mgKOH/g	<0.5	<0.5	1.1	0.3	0.003	0.266
Methanol or ethanol	%, m/m	–	≤0.2	0.3	n/a	–	–
Distillation	°C	≤360	n/a	360	352	–	–

[a] mg/kg.
[b] ppm.

The development of mass-rearing systems of insects opens the door for their utilization as a source for biodiesel. Studies have shown different fat content and composition depending on the development stage and species. So, the wide diversity of insects could result in a large variety of possible biodiesel sources. However, their potential as biodiesel has rarely been studied, and the characteristics and properties of their lipids as biodiesel are little-known.

Fuel quality is defined by a set of parameters concerning characteristics of heat, fluidity, purity preservation and generation of waste. In addition to this, it also must be compatible with current biodiesel engine technology. Biodiesel, when it is produced by transesterification, is a mixture of fatty acid esters whose fuel properties are influenced by the type of alcohol and fatty acids from which they are derived [84]. Therefore, different types of fat will provide different qualities of biodiesel. In the case of livestock (pigs, cattle, sheep, goats), though the fat percentage varies between races and species, there are no big differences in the fat quality and composition. In insects, however, significant variations in fatty acid composition have been found, depending on the taxonomic group studied [85,86], the stage of development [87,88] or the food source [89], an example of this point is given in Table 7. This means that the selection of these variables or the oil mixture obtained from different species can allow some degree of manipulation of the quality of the fat obtained in order to adapt it to some specific purposes.

Although there are few publications on the quality of insect fat for biodiesel production, the composition and percentage of fatty acids from the fat of some insect species could provide guidance for the quality of that fat for biodiesel production. Specifically, in the case of high heating value, it has been found that the theoretical estimate of this value based on the mixture, percentage and heat value of each fatty acid is consistent, with a very small percentage of error, with high heating value determined experimentally [89].

In this regard, it was observed that the cetane number ($C_{16}H_{34}$), which indicates the ignition capability and, therefore, the quality of the biodiesel, diminishes with decreasing chain length and increasing branching [84] unless the ester is derived from alcohol [90,91]. Insect fat is rich in fatty acids of more than 16 carbons, especially those with between 16 and 18 carbons [84,90]; fatty acids with this number of carbons have a cetane number between 66 and 99 in the case of saturated fatty acids and between 50 and 70 in the monounsaturated fatty acids [78], also very abundant in insects. In both cases, the minimum cetane number required for fuels (42 according to [68]) is exceeded.

The range of heat of combustion is another parameter in the quality of biodiesel. In fatty acids, it increases with the number of carbons [92–94]. The heat of combustion generated by the majority of fatty acids in insects, with 16 or more carbons, is found to be between 2550 and 3012 kcal/mol [92–94], being similar or greater than the heat of combustion of hexadecane (cetane), which is 2559.1 kcal/mol [94].

The problems with this type of biodiesel are mainly related to density, viscosity, fluidity and lubrication. The viscosity range of 16–18 carbon fatty acids [84] is within the range established for diesel, which is between 4 and 5 [95,96].

In general, for fatty acids, viscosity increases with the number of carbons and degree of saturation. In unsaturated fatty acids, the position of the double bond seems to affect the degree of viscosity much less than the configuration of the double bond, presenting a higher viscosity in the trans configuration than in the sis configuration (5 in [90] FA fuel quality). Another problem associated with biodiesel is the low fluidity, especially at low temperatures, which creates lubrication problems. Esters derived from alcohol, such as isopropyl and isobutyl, have the advantage of maintaining their properties at low temperatures [84]. Moreover, the presence of unsaturated fatty acids such as linolenic and, principally, linoleic acid, or their esters, with a viscosity range between 2.65 and 3.64 [84], can contribute to improving viscosity at low temperatures.

Oxidation capacity influences the presentation of fuel. The oxidation capacity of fatty acids is exponentially related to the degree of unsaturation. While the oxidation capacity of derivatives of oleic acid is 1, and those of linoleic and linolenic acids are 41 and 98, respectively [97], means that small amounts of HUFAs can disproportionately affect fuel quality. In this way, fats from some – especially aquatic – insect species [86], present certain amounts of HUFAs that can negatively affect biodiesel conservation. However, terrestrial species such as mole crickets, ground crickets and spur-throated grasshoppers lack fatty acids with more than three double bonds [98].

In order to compare the properties of biodiesel from insects and other plant origin, such as rapeseed, an example is shown in Table 8, which compares with the European (EN14214) and American (ASTM D975) standards for biodiesel.

8. Conclusions

In this review, we have demonstrated the fat potential of insects and provided the principal criteria to be considered for selecting insect species for biodiesel production.

It was observed that fat content varies widely between orders, species, and their stages of development – larva, prepupa, pupa, nymph or adult – with the larval stage being that at which the most fat is accumulated. Furthermore, variations in the fat content were also observed within the same species due to factors such as origin (wild or bred in captivity) or type of diet. This last factor is one of the most important to take into account for the selection of insect species with the objective of using their fat in the production of biodiesel.

Saturated fatty acids C16 and C18, which have a high calorific value and good potential viscosity, are the fatty acids with the

greatest potential for biodiesel production. They are present in large amounts in terrestrial insects.

Therefore, insects can be a renewable source of protein and energy, since through the development of their life cycle they can be fed with agricultural, industrial or urban by-products in order to accumulate a large amount of fat for conversion into energy through biodiesel production. Moreover, the resulting protein can also be used as a protein source in animal feed.

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